GLYCOENZYMES: A NOTE ON THE ROLE FOR THE CARBOHYDRATE MOIETIES*

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Summary: The carbohydrate moieties of the glycoenzyme, glucoamylase I from Aspergillus niger, are linked by O-glycosidic bonds to approximately 45 serine and threonine residues, presumably on the surface of the enzyme molecule. The glucoamylase is remarkably stable on storage at low temperatures. Extensive oxidation of the carbohydrate residues in the enzyme by periodate markedly affects the stability of the enzyme. It is suggested that the carbohydrate moieties function as stabilizers of the tridimensional structure of the glycoenzyme, and, in turn, of the catalytic property of the molecule.

With the many improvements in the techniques for enzyme purification and analysis, it has become evident that many enzymes contain covalently linked carbohydrate residues as components of their molecular structures. In these enzymes, the carbohydrate portion most often consists of mannose, glucose, galactose or glucosamine in arrays characteristic for each enzyme. The term glycoenzyme has been suggested as appropriately descriptive of enzymes of this type (1). Most recent examples of glycoenzymes are bovine pancreatic deoxyribonuclease (2), plant alpha-galactosidase (3), and yeast invertase (4) while earlier examples include ribonuclease B (5), chloroperoxidase (6), glucoamylase (7) and glucose oxidase (8). An intriguing aspect of glycoenzymes pertains to the function or functions of the carbohydrate residues at the molecular level. An earlier proposal (9) that the carbohydrate residues are essential for the transport of the enzyme through cellular membranes is indeed

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plausible but difficult to verify experimentally. A second proposal that the carbohydrate moieties protect the polypeptide chain of glycoproteins and, presumably glycoenzymes against proteolysis (10) has been substantiated by experimental data (11). In this communication a third proposal is advanced for the role of the carbohydrate residues, specifically that these residues function as stabilizers of the tridimensional structure of the protein moiety of the glycoenzyme. This suggestion is made on the basis of new information on the structure of glucoamylase I, a glycoenzyme from Aspergillus niger, and the finding that the native enzyme but not periodate-oxidized enzyme is remarkably stable.

Aspergillus niger produces two forms of glucoamylase (12) both of which are glycoenzymes containing mannose, glucose and galactose (7). The more abundant form, glucoamylase I with approximately 80 residues of mannose, 20 residues of glucose and 2 residues of galactose per mole of enzyme, has been used in the present studies. Samples of highly purified glucoamylase I were subjected to alkaline borohydride reduction for 4- or 6-day periods at 0° at reaction conditions which effect a reductive removal of a high percentage of the carbohydrate residues from the protein (13). Under the alkaline conditions of the reduction, the enzyme was also degraded and enzymic activity was lost. The reduced and native enzyme preparations were then analyzed for tryptophan by a spectrophotometric method (14) and acid hydrolyzates of these preparations were analyzed for other amino acids by cation exchange chromatography.[a] The molecular weight of glucoamylase I was found to be 110,000 from density gradient centrifugation data (15, 16) and this value was used for calculating the number of amino acid residues per mole of enzyme (Table 1). Appropriate correction factors based on loss of carbohydrate as measured colorimetrically (17) were used to obtain the values for the borohydride-

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reduced enzyme and for the destruction of serine and threonine (18) during acid hydrolysis of the enzyme.

It should be noted in Table 1 that a reasonable balance in the loss of serine and threonine and the gain in alanine, glycine and α-amino butyric acid was obtained. In the borohydride reduction and glycosyl elimination reactions, serine is reduced to alanine, the threonine is reduced to α-amino butyric acid or degraded to glycine (13, 19), while the carbohydrate residues which are attached to the amino acids are reduced to the sugar alcohols (20). Other amino acid values remain relatively unchanged and are within the experimental error of the analysis.

In order to check for a possible influence of the carbohydrate residues on the stability of the enzyme, appropriate samples of the purified glucoamylase I were subjected to mild periodate oxidation in 0.02 M sodium periodate of pH 5.0 for 6 hours or 24 hours at room temperature.[b] Under these conditions approximately one-third of the carbohydrate residues were oxidized in the 6-hour period as determined by a colorimetric method (17) and two-thirds in the 24-hour period. Following dialysis of the 6-hour sample against distilled water at 4° for 24 hours and enzyme assays by a standard procedure (21) it was found that the total and the specific activities of this sample were not changed significantly. However in the 24-hour sample, a white amorphous precipitate, presumably denatured enzyme, had formed and assays of appropriate samples of this material showed that less than half of the original activity was retained. When native glucoamylase I was stored in phosphate-citrate buffer of pH 4.5 at 4° for 6 years or in the frozen state for periods of 1/2 and 1 1/2 years and assayed for enzymic activity, it was found that 94%, 98% and 100% of the original activity was retained in these solutions. Quite

[b] The use of periodate oxidation for this purpose was first suggested by K. Kleppe (7), presently at the Enzyme Institute, University of Wisconsin, Madison, Wisconsin.

clearly the native glucoamylase is remarkably stable in contrast to periodate-oxidized enzyme. It is suggested that this stability is attributable to a specific role of the carbohydrate moieties on the tridimensional structure of the enzyme. From reports in the literature (22, 23), it would appear that carbohydrate residues also impart stability to other carbohydrases as, for example, invertase. However, another type of glycoenzyme, chloroperoxidase (24) has been found to retain full catalytic activity even when 90% of the carbohydrate has been removed from the protein. The differences in stability among various glycoenzymes may be due to differences in the arrangement of the carbohydrate residues on the protein portion of the molecule.

The following conclusions are advanced on the basis of the new findings on glucoamylase and on the basis of the known pathways for the biosynthesis of glycoproteins (25). The carbohydrate residues of glucoamylase are linked by O-glycosidic bonds to serine and threonine residues of the polypeptide chain of the enzyme. In view of the fact that the glucoamylase contains approximately 100 carbohydrate residues per mole of enzyme and that there are approximately 45 points of attachment of the carbohydrate residues to the serine and threonine of the polypeptide chain (Table 1), the average length of the carbohydrate chains in the enzyme is 2.2 residues. Obviously, this calculation does not allow for other types of linkages of carbohydrates to other amino acids of the protein. Nevertheless, the data do indicate a unique molecular architecture for the glucoamylase which together with the stability data is the basis of the proposal that carbohydrate moieties function in the maintenance of the conformational structure of glycoenzymes.

A glycoenzyme with this type of molecular architecture is probably synthesized in the intact fungal cell by the following sequence of molecular events. First, the polypeptide chain is assembled on the ribosomes as directed by the appropriate messenger RNA, transfer RNA and associated factors; second, this polypeptide chain dissociates from the ribosomes and folds into proper conformation as determined by the bonding forces between the

amino acid residues of the polypeptide chain; and third, the carbohydrate residues are attached to the serine and threonine residues on the surface of the enzyme molecule via nucleotide diphosphate hexose and appropriate glyco-

Table 1

Number of amino acid residues per mole of glucohydrolase I before and after reduction in alkaline sodium borohydride

	Before[c]	After[d]	Ratio
Aspartic	93	97	1.04
Threonine	119	91	0.76
Serine	132	115	0.87
Glutamic	65	66	1.02
Proline	33	32	0.97
Glycine	66	79	1.20
Alanine	90	115	1.28
Cystine/2	8	4	--
Valine	55	57	1.04
Methionine	4	4	--
Isoleucine	32	34	1.06
Leucine	61	65	1.06
Tyrosine	34	35	1.03
Phenylalanine	32	33	1.03
Lysine	19	17	0.91
Histidine	6	6	--
Arginine	25	24	0.96
Tryptophan	33	34	1.03
α amino butyric	0	8	∞

[c]Average of five determinations on four preparations
[d]Average of three determinations on two preparations

syltransferases (1, 25, 26). The hypothesis on the role of carbohydrate in maintaining a tridimensional structure of the glycoenzyme is equally valid even if the synthesis of the glycoenzyme is completed in subcellular particles such as the mitochondria or Golgi particles from precursor glycopeptides (25, 27). Also, the possibility that the carbohydrate residues become attached to the polypeptide chain prior to folding of the molecule is of course not excluded. In this case the carbohydrate residues would influence the manner in which the glycoenzyme folds into its characteristic conformation as well as influencing the stability of the intact enzyme.

Several types of experiments are in progress for obtaining additional information on the proposed structure for the glucoamylase and for testing the suggestion that the carbohydrate residues function as stabilizers of the conformational structure of the glycoenzyme. One experiment involves stripping the carbohydrate residues from the glucoamylase by a procedure based on periodate oxidation (24) and determining the stability properties of the enzyme devoid of carbohydrate moieties.

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